



STUDIES ON THE FALLOPIAN TUBE OF ALBINO RAT

Dissertation submitted in partial fulfilment of
the requirements for the degree of
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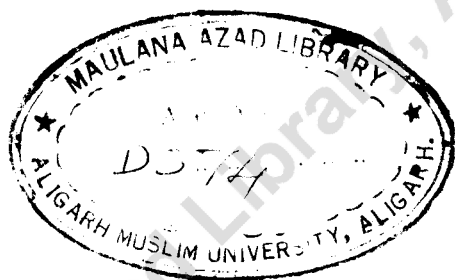
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By

NARGIS NAZEER USMANI

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DS74

Humayun Murad
Ph. D.

Department of Zoology
Aligarh Muslim University
ALIGARH-202001 (INDIA)

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This is to certify that the dissertation for M.Phil. in Zoology has been completed by Miss Nazeer Usmani under my supervision. It is original in nature and I have permitted the candidate to submit it for the award of M.Phil. degree in partial fulfilment of the M.Phil requirements in Zoology.

Humayun Murad
(Humayun Murad)
Lecturer
Department of Zoology,
Aligarh Muslim University,
Aligarh.

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HISTORICAL REVIEW

Reproduction is the mechanism by which a species maintained from generation to generation. In mammals the process of reproduction is sexual. The procedure through which it is accomplished is, in most cases, a very complex and intricate process. The development of a multicellular animal is prefaced by the formation and maturation of the germ cells. Which will unite to form the zygote or fertilized egg. The zygote carries the parents entire hereditary contribution. The germ cells are produced by the gonads, or sex glands, of the male and female parents. The male germ cell, the spermatozoa, is produced in the testis; the female germ cell or ovum is generated within the ovary.

Reproduction can be conveniently divided into five phases or parts: gamete formation, fertilization, implantation, gestation and parturition. A detailed information on mammalian reproduction has been given by Asdell (1928), Nalbandov (1958) reported that the reproductive cycle of mammals, in its broadest sense, including maturation and functioning of the gonads, the associated sexual organs and the secondary sexual characteristics, is controlled by interaction of series of hormones. Each of these hormones are specific in its activities, starting (or stopping) specific functional activities of specific

organs. Optimal reproductive performance does not occur, however, unless the whole endocrine system is in proper balance. Several reviews of physiology and light-microscopic morphology of the reproductive system has been published viz. Austin (1963); Blandau (1961); Eckstein and Zuckerman (1956).

Most mammal have breeding season restricted to a definite time of year. This breeding season is usually at a time most advantageous for the pregnant female and the new born young. Even in those mammals which breed through out the year, there is usually some period of lesser activity associated with period of environmental stress. According to their breeding pattern all species of animals can be grouped into two categories i.e. seasonal breeders and continuous breeders. The seasonal breeding includes species in which the gonads of both the sexes regress completely and become inactive; in other species, however, only the female become periodically sexually inactive, the male showing continuous spermiogenesis, which appear to be independent of season. In species that have become continuous breeders, more or less distinct peaks of prolificacy are discernible. There is no complete agreement whether reproductive efficiency varies seasonally in laboratory rats, which are also continuous breeding species. Corbin and Schottelius (1960) however, reported high fertility rate during spring and summer. The copulation only permitted during a definite period of time

(Eckstein and Zuckerman, 1956). These periods of proper psychological and physiological state, during which copulation is permitted are called period of 'heat' or estrus. The psychological and endocrine events are all correlated while the main physiological events of the estrus cycle are occurring in the ovaries, these events are reflected in the changes that take place in the uterus and vagina under the influence of the ovarian hormones - estrogen and progesterone. The rats in which cycle lasts about four days, very careful examination have been made between ovarian morphology and histology of the uterus and vagina (Asdell, 1946; Reynolds, 1949).

The fallopian tube is an accessory reproductive organ of the female. It is the most important part of the reproductive system as it provides a suitable place for the union of the gametes and formation of the blastocysts.

Contemporary knowledge of the mammalian oviduct derives in great part from twentieth century advances in technology and the application of endocrinological concept and techniques. Yet long before our time mammalian oviduct was identified, its structure described and its role in generation defined.

G. Fallopius made most notable contribution to anatomy. His contribution which concerns most are correct and accurate description of the female reproductive system including the

oviduct which today are known as the "Fallopian tube". Gabriele Fallopius published the first anatomical description of a mammalian oviduct in 1551 (Woodruff and Paurestein, 1969). Henle (1873) has given little more details of the fallopian tube than Fallopius, later William (1891) gave still more detailed description of the intricacies of the oviduct. Gradually the tube of the Fallopius acquired three more names, oviduct, uterine tube and salpinx.

Although the oviduct has been distinguished as a structural identity since the sixteenth century, knowledge of its functional significance has remained obscure. The original concept that the oviduct is a simple pathway between the ovary and the uterus has been abandoned. The oviduct is not only a conduit where ova and p spermatozoa unite but also an active participant in gamete transport (Paurestein et al., 1968), fertilization (Hamner and Fox, 1969), maturation and development of embryo (Glass, 1969). These functions are probably affected partially through the oviducal fluid secreted by the oviducal epithelium.

The transport of ovum through the oviduct is not continuous. At first the ovum is rapidly transported by ciliary activity through the infundibulum. It passes through the ampulla at a slower rate and is then arrested close to the ampullar - isthmic junction for about 24 hours these after for the next day or two, it is transported slowly through the

isthmus portion to the uterine cavity (Blandau, 1969).

The emergence of the oviduct as reproductive organ having transport and secretory function which may be essential for reproduction has stimulated interest in oviducal function and their control mechanism. The first comprehensive review of the mammalian oviduct and its function a multiple disciplinary point of view was published in 1969 by Hafez and Blandau (1969), Woodruff and Paurestein (1969) and recently by Johnson and Foley (1974).

Several review of the physiology and light microscopic morphology of the oviduct has been published, Austin (1963); Blandau (1961); Eckstein and Zuckerman (1956). The few published papers on electron microscopy of the oviduct has been compiled by Horstman (1966). Fredricsson in a series of work (1957, 1959, a, b, c) and Fredricsson and Bjorkman (1962 and 1968) have traced the histochemical patterns of the tubal epithelium. Moghissi (1971) and Fergelson (1972) have studied the protein patterns of oviducal fluid. Friedly and Seymour (1975) have observed histochemically and biochemically, carbonic anhydrase activity in the oviduct.

Regional differences in the rabbit fallopian tube has been emphasized by Gupta et al. (1967, 1969, 1970, a, b), Roy et al. (1971). The ciliated cells of the fallopian tube play an important role in ovum transport (Shipstone et al.,

1974a,b). The biochemistry and morphology of the secretory cells have been studied by Shipstone et al. (1974b).

While it is widely accepted that the oviduct plays a primary role in reproduction, there is wide range of opinion about what this role is. Most of the work on fallopian tube is confined to biochemical and histochemical studies, but there is paucity of information on histological differentiation in different segments of the tube.

The present study is aimed to fill the lacunae in the knowledge on the fallopian tube in normal condition as well as in presence of IUCD. The present study is aimed at defining the histological pattern in fallopian tube to determine how far this information can be utilized for altering the environment of the tube with the view to making it less suitable for fertilization as well as maintenance of the egg.

ANATOMY OF THE FEMALE REPRODUCTIVE
SYSTEM OF RAT

INTRODUCTION

The two cardinal concern of living things are the maintenance of self and the perpetuation of the species. Reproduction is essential so that the species may continue to live upon the earth. Sexual or gamic reproduction is the rule in the phylum chordata. It involves the union of the gametes, or reproductive cells, of male and female to form a zygote or fertilized egg under proper condition and by a series of complicated process the zygote develops into an embryo.

The reproductive organs differ from all other organs of the body in that their functions, do not contribute primarily to the welfare of the individual of which they are a part, their chief concern lies with the succeeding generation. The organs of the reproductive system are divided into essential and the accessory. The essential organs are the gonads which are concerned with the formation of the germ cells and with the secretion of the hormones which control the activity of the accessory sex organs and maintain the secondary sex characters. The accessory sex organs include those structures needed for the transmission and transfer of the germ cells and the development of the fertilized egg into a new individual. The accessory

reproductive organs of the female are the uterine tube (fallopian tube), the uterus, the vagina, the urogenital sinus, Bartholin's glands, the clitoris, and the mammary glands.

The regulation of a normal reproductive cycle is dependent upon an interplay of uterine, oviducal, ovarian, pituitary and hypothalamic function. An essential feature in this balanced regulation is the hormonal stimulation of morphological and biochemical transportation within the genital tract of all mammalian species, including the human being. The peripheral target tissues of anterior pituitary gonadotrophins and ovarian steroids provides the bases for normal oocytes maturation, fertilization and early development of the cleaving embryo, the blastocysts and its implantation, by means of follicular, oviducal and uterine proliferation and secretion.

The ovaries are paired organs situated caudate to the kidneys. They remain near the kidney, where they are first differentiated but they do not undergo the elaborate descend that is typical of the testes. The size of the ovary depends largely on the age and reproductive state of female because the growth of the ovary and the development of its histological components are controlled by hormones from the pituitary gland (Pettkoi, 1944). Nalbandov (1958) suggested that the shape

of the ovary varies greatly with the species and depends largely on whether the female are habitually polytocous (litter-bearing) or monotocous (Single-bearing). But the ovaries are best described as roughly bean shaped in most mammals, although they are greatly elongated in rabbits. The surface in most mammals is irregular, especially during the breeding season when follicles and corpora lutea frequently protrude (Asdell, 1946a). The two most important components of the ovary are the follicle and corpus luteum. Follicle are produced by the action of pituitary hormones (Hammond, 1927). Asdell (1946b) reported that the follicles mature mainly during estrous.

The paired connecting fallopian tube between the ovaries and uterus are long convoluted derivatives of the mullarian ducts. The degree of convolution varies between species. In some of them (rabbit) the duct is almost straight while in others (woman) it shows extreme convolution (Lee, 1928). Fallopian tube serves as a mean by which spermatozoa may travel from the uterus towards the ovary and for the transport of the ovum into the uterus. The oviduct is suspended by mesosalpinx, a derivative of the broad ligament. The opening of the oviduct is termed "ostium" is either fimbriated or bears along its internal surface numerous radiating ridges. In most species the fimbriae form a fringed open funnel which envelops the ovary more or less completely. In rats and mice it forms

a sac around the ovary and is then called the bursa ovarii. In these species the bursa is perforated by a small tube. Anderson (1927) made the following classification of fallopian tube, according to the type of the uterus.

(i) In marsupial the isthmus is tortuous, and has a wide lumen which narrows down somewhat at the point of entry into the uterus. This is neither a sphincter nor folds.

(ii) In all animals with bicornuate uteri, the isthmus is either straight or tortuous and always has a thick muscular coat and narrow lumen.

(iii) In animals with simplex uterus, the oviduct opening has protected villi or folds, although Williams (1891) states that in woman the uterine ostium has a sphincter.

For convenience and descriptive purposes the oviduct is usually divided into 4 segments, which can be distinguished anatomically.

1. Preampulla.- Thin walled mainly concerned with transport of egg from the ovary into ampulla.

2. Ampulla.- With slightly thicker wall where mostly fertilization occur.

3. Isthmus.- Thick walled, affects the nutrition and transport of the sperm.

4. Juncture. - Well developed thin walled, narrow lumen of the whole oviduct.

The uterus usually consists of two horns and a body. The body / or horns of the uterus are suspended bilaterally by the broad ligament which originate from the dorsal lateral wall of the pelvic cavity so that the uterus hangs suspended in the peritoneal cavity. Anderson (1927) reported four basic types of the uterus occurring in mammals:-

(a) Duplex uterus:- In this arrangement, the uteri are completely separate, each with a separate cervix or opening from the vagina, but are joined to a single vagina.

(b) Bipartite uterus:- Single cervix, lower portion of the two uteri are fused, but in the major part of their length the uteri are still separate.

(c) Bicornuate uterus:- In this arrangement, the lower two-third of the two uteri are fused into a single structure while the upper third of each uterus remain separate.

(d) Simplex uterus:- The uteri are fused into a single structure so that, functionally there is but a single uterus.

Cervix is a sphincter muscle that lies between the uterus and vagina. The main function of the cervix is to close the uterine lumen against microscopic and macroscopic intruders. The cervical canal is closed at all the times except during parturition.

The vagina is a canal which extends caudate from its circumference of the neck of the uterus to the level of the ascending ramus of the ischium. Vagina can be divided into two parts: The vestibule (outer most part of the vagina) and the posterior vagina (extending from the urethral opening to the cervix). The external genitalia comprise the clitoris, the labia majra, and labia minora; and certain glands that open into the vaginal vestibule.

In present work the efforts have been made to study the anatomy of the female reproductive system of the rat in different regions.

MATERIALS AND METHODS

The albino rats used in the experiments were derived from a closed colony. These rats were kept under proper laboratory condition. For experimental purpose two to three months old female rats were chosen and then they were maintained in isolation without males. After a period of time, these rats were killed, a mid ventral incision was made and the whole reproductive system exposed for examination. The separated out reproductive system were then kept in glycerine for detailed anatomical observation during prepubertal and postpubertal stages.

RESULTS

The female reproductive system of rats includes:

- 1- Ovaries, 2- The fallopian tube or oviduct
- 3- The uterine horns, 4- The cervixes, 5- The vagina,
- 6- The external genitalia.

THE OVARIES.

The ovaries are paired organs which lie one on either side of the abdominal cavity, shortly caudate of the kidneys. These are 5.8 cm in front of the valvular opening. The ovary is suspended in the abdominal cavity by a fold (mesovarium) of the cranioventral portion of the broad ligament. From the caudal extremity of the ovary there arises a strong ligament by which it is attached to the uterine horns. The latter ligament is called the ovarian ligament. Each ovary is characteristically berry shaped. The size of the ovary depends largely on the age and reproductive state of the female. These usually are 5 mm long, 4 mm deep and 4 mm wide, each weighs 32-20 mg. In older females an increase in ovarian weight has been noted. The surface of the ovary is irregular especially when follicles and corpora lutea protrude. Puberty does not cease the growth of the ovary.

THE FALLOPIAN TUBES.

The oviduct, or fallopian tube, is the paired convoluted tubule that is derived from the upper portion of the mullerian duct. They reach from the ovary to the tapered ends of the uterine horns. The tube lies in a peritoneal fold derived from the lateral layer of the broad ligament. The fallopian tube measuring about 1-1.5 cm in length in prepubertal and 1.5-2 cm in virgin female. The ovarian end of an oviduct is flared out into a fimbria, or funnel with fringed edges. This end forms a complete capsule, which encloses the ovary as in a sac. This sac is called the bursa ovarii, a small perforation is present in one wall of the bursa. At the time of heat and ovulation the fimbriated end of the oviduct shows great motility, which probably helps the egg to find its way into the oviducts. On the basis of their diameter and internal structure, the fallopian tube is divided in the following divisions.

(i) The Preampulla:- The preampulla comprises the fimbriae and infundibulum, it consists of 1-2 loops. This is thin walled structure and mainly concerned with the transports of egg from the ovary into ampulla.

(ii) The ampulla:- Next to the preampulla is ampulla. The ampulla constitute 3 loops, also the part with largest diameter. Slightly thick walled, fertilization occurs here.

(iii) The isthmus:- The ampulla tapers down to the isthmus. The isthmus forms a long and coiled part of the oviduct consists of the 4th to 9th coils. This is the narrow portion of the duct which adjoin the uterine horns. It is thick walled, affects the nutrition and transport of sperms.

2. Juncture:- The junctura constitute the last loop of the extramural and intramural portion of the fallopian tube. The external loop rests on the surface of the uterus and bends sharply toward the antimesometrial side of the uterine wall. This is the narrowest portion of the fallopian tube. This is the area of transition between the oviduct and uterine horn.

3. The uterus:- The uterus of the rat, like that of most other rodents and rabbits is a duplex structure, the uterine horns are completely separate each with a separate cervix or opening from the vagina, but are joined to a single vagina so that the uterine body is absent. The uterine horn gradually taper and joins the oviducts. The length of the uterine horns are usually 3.5-3.8 cm. This length varies greatly with the age of the animal and with other factors. The uterine horns usually lies in pelvic cavity or in abdominal cavity. These are attached to the pelvic and abdominal walls by the broad ligament.

4. THE CERVIXES.

The cervix lies between the uterine horn and the vagina. They are two in number. The cervix is the thick-walled portion of the reproductive tract which extends for 5 mm from its junction with the uterine horns to the thin-walled vagina. The lumen of the cervix is interrupted by transverse interlocking ridges called annular rings, this makes possible tight closure of the cervix with a cervical plug that is formed during pregnancy. Immediately before parturition this plug liquefies and shortly thereafter the cervix relaxes.

5. THE VAGINA.

The vagina is a thin-walled structure which extends from cervix to the point of junction of the urethra. The vagina can be divided into two parts: the vestibule and posterior vagina. In non-pregnant normal female the vagina and the vestibule together is 5 mm long. During pregnancy, the vagina may be twice as long.

6. THE EXTERNAL GENITALIA.

The external genitalia of the female rat consists of clitoris, the labia majora and labia minora and bulbo vestibular glands or Bartholin glands. The clitoris is the embryological

homologue to the penis. The outer genitalia are well supplied with sensory nerve endings, which play an important role during sexual excitation of the female. The clitoris is capable of limited erection during the sex act and the labia, because of an increased blood flow, become extremely turgid.

DISCUSSION

The female rat not only contribute the female sex cells essential for starting a new individual, but also provides the environment in which the new individual is conceived and nourished during the early days of its life. These functions are carried out by the primary and secondary organs of reproduction. The primary organ of the reproduction the ovaries, they not only perform the important function of producing egg cells but also produce hormones. Each of these hormones are specific in its activities starting (or stopping) specific functional activities of specific organs. These hormones prepare the reproductive tract for a pregnancy and help in maintaining and delivering the products of the pregnancy. Nalbandov (1961) suggested that the growth of the ovary and development of its histological components are controlled by the hormones from the pituitary glands. The size of the ovary depends largely on the age and reproductive state of the female. The shape of the ovary varies greatly in same individual, Blandau (1961) studied and proposed that it depends largely on whether the females are habitually litter bearing or single bearing. Cole et al. (1930) reported continuous formation of new, potential ova throughout adult life. The secondary organs of reproduction comprise the

oviducts or fallopian tubes, the uterus, the cervix, the vagina and external genitalia. The oviduct or fallopian tube of rat, is convoluted tubule that is derived from the upper portion of the mullerian duct. The degree of convulation varies between species. In some of them (rabbit) the duct is almost straight while in others (man) it shows extreme convulation (Lee, 1928). Lee (1925 and 1928) observed that the opening of the oviduct is always guarded by special folds. According to the structure of the epithelium 4 segments of the oviduct may be distinguished, the preampulla, ampulla, the isthmus and the junctura. Anderson (1927) has also made the following classification of oviducts according to the type of uterus present.

1. In marsupials the isthmus is tortuous and has a wide lumen which narrows down somewhat at the point of entry into the uterus. There is neither a sphincter nor folds.

2. In all animals with bicornuate uteri, the isthmus is straight or tortuous and always has thick muscular coat with narrow lumen.

3. In animals with simplex uterus, the oviduct opening has not protected villi or folds. The uterus is a bicornuate structure, consisting of two completely separated uterine horns, with two cervixes and no uterine body. Some animals, including the rats, normally show a regularly recurring estrous

cycle throughout their reproductive life. Nalbandov (1958) and Rowan (1938) point out that numerous investigations have shown that reproductive rhythm depends on pituitary activity. The stimulus causes the pituitary to release gonadotropic hormones. Action of light, via the eye and optic nerve, on the pituitary gland. Mercier (1947); Hammond (1927) are also of this thought. Hammond (1927) has cited the presence of large follicle during prepubertal stage. Asdell (1946b) reported that the maturation of the follicle occurs during estrus. Taylor and Weber (1951) described the rupturing of the follicles as a result of increased intrafollicular pressure or enzymatic dissolution. Usually fertilization occurs shortly after ovulation. The regulation of a normal reproductive cycle is dependent upon an interplay of uterine, oviducal, pituitary and hypothalamic function. An essential feature in this balanced regulation is the hormonal stimulation of morphological and biochemical transformations within the genital tract of all mammalian species, including the human being.

HISTOLOGY OF THE FALLOPIAN TUBE

INTRODUCTION

The problem of the human fertility and sterility has always attracted considerable interest. The attention has been chiefly directed to the ovaries and the uterus, and much information has been gained through these studies. However, the fallopian tube with their intermediate position have been more or less completely disregarded and have frequently been ascribed a transport function only. The relative lack of information about this organ applies to all branches of study including the morphology, histology, biochemistry and histochemistry. The emergence of the fallopian tube as reproductive organ having transport and secretory function which may be essential for reproduction has stimulated interest in oviducal function and their control function.

Most of the work on fallopian tube is confined to biochemical studies but there is paucity of information on histological differentiation in different segment of the tube. The objective of this work is to gain a deeper understanding of the events occurring in the histology of the fallopian tube under the normal condition as well as in the presence of intra-uterine-foreign-bodies.

The fallopian tube or oviduct in mammals is an accessory reproductive organ, it provides suitable system to support the varied processes associated with the initiation of new life. Among these are fertilization and the events which precede it, and properly engineered sperm and ovum transport. Some animals including the rat, normally show a regularly recurring estrous cycle throughout their reproductive life. Such animals are said to be polyestrous. This rhythmic change of the female reproductive system is called the estrous cycle. The high-light of this cycle is the presence of 'heat' during which the female receptive to the male, and soon thereafter the egg is shed. The duration and intensity of the heat are variable. When in heat, many animals show a greatly increased activity. Rats in cages run spontaneously much more at the height of heat than during diestrus or after castration. The estrous cycle can be divided roughly into four periods according to certain visible and invisible changes that occur during the cycle (Duke, 1942; Asdell, 1946).

1. Proestrus:- Period of preparation.
2. Estrus:- Period of desire.
3. Metestrus }
 } Period of sudden cessation of heat.
4. Diestrus }

Several reviews of the physiology and light microscopic morphology of the reproductive system have been published Austin (1963), Blandau (1961), Eckstein and Zuckerman (1956). The first comprehensive review of the mammalian oviduct and its function a multidisciplinary point of view was published by Hafez and Blandau (1969); Woodruff and Pauerestein (1969) and recently by Johnson and Foley (1974). A few published papers on electron and light microscopy of the fallopian tube have been compiled by Horstman and Stegner (1966). Light and electron microscopy of oviduct has revealed that there are greatest differences than expected in the fallopian tubes among varied segments and in the same segment of a fallopian tube under various hormonal condition. Therefore, the present review will be confined mostly to oviducal studies by light microscopy.

The rapidly increasing use of the intra-uterine device (IUD) for family planning purposes has stimulated considerable research on its biological effects in experimental animals and human beings. Several comprehensive reviews of the subject have appeared in recent years (Basic and clinical aspects of intra-uterine devices) Report of WHO (1966). The research shows that IUD has an antifertility effect in every species tested but that the stage of the reproductive process influenced by their presence differs from species to species. In spite of numerous investigations, the mechanism of action of IUCD is

poorly understood and the picture that has emerged so far is complex and confusing. There is no evidence of a systemic effect; the antifertility action is confined to the horn bearing the foreign body. Treated animals have normal pituitary content of gonadotropins (Chaudhary, 1965), normal estrous cycle and normal corpus luteum function (Marston and Chang, 1964). The ovulation sperm transport, fertilization and zygote transport through the oviducts occur normally. Earlier observations suggest inhibition of ovulation (Nalbandov, 1958) but more recent works indicate that ovulation is not affected by the presence of intra-uterine threads and the eggs picked up by the oviduct is somewhat inhibited. It appears that the ova transport through the oviduct is not affected but that blastocysts degenerate or are prevented from implanting where they enter the uterine cavity (Kar et al., 1964). The biochemical composition of tubal fluid does not show any noteworthy alterations in the presence of an intra-uterine foreign body in rabbit (Kar et al., 1965).

Among the experimental animals, the rat has been most extensively used for the study of the biologic action of an intra-uterine foreign body. An antifertility effect occurs, which is reversible upon removal of the foreign body. There is no evidence of a systemic effect; the antifertility action is confined to the horn bearing the foreign body. No information is available on the effect of an IUCD on the morphology,

morphology, histology, ultra structure or histochemistry of the tubal tissue. This is true also for the tubal fluid milieu. Therefore, it seems appropriate to begin a discussion on the fallopian tube with a description of the histology of the rat oviduct in normal condition as well as in the presence of intra uterine foreign body.

The finding presented in this work should help to demonstrate the necessity for an approach to histology of the fallopian tube of rat and that we need much more intense effort in this area of reproductive research.

MATERIALS AND METHODS

Rats were selected for this study were derived from a closed colony, kept under laboratory condition and fed on standard food obtained from Lever brothers. Two to three months old virgin females were selected and maintained without males unless mating required.

Experiment No. 1:-

For histology (light microscopy) of the fallopian tube in normal conditions, specimens from different parts of fallopian tubes including the infundibulum, the ampulla, the isthmus and uterotubal junction were taken surgically

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D.P.X.

for observation under light microscope.

Experiment No.2.

Another group of 12-16 weeks old, normal cyclic virgin females were selected and maintained without males. Under anaesthesia their reproductive systems were exposed through a mid-ventral incision. A copper wire of 0.2 mm in diameter and 2 cm in length was unilaterally inserted into the left uterine horn. After two weeks recovery period, the rats under experiment were sacrificed and the tissues from the fallopian tube of contraled horn and Cu-IUD bearing horn were taken out. These tissues were fixed in Cornoy's fluid which is proved good both for cytoplasmic as well as nuclear differentiation. 5 μ thick sections were prepared from each portion of fallopian tube, dehydrated and stained with haemotoxyline, counter stained with alcoholic eosin, sections were dehydrated and mounted in D.P.X. for microscopic observations.

RESULTS

The oviduct, or fallopian tube (Flt) is the convoluted tubule that is derived from the upper portion of the mullarian duct. It serves as a means by which spermatozoa may travel from the uterus towards the ovary and for the transport of the ovum into the uterus. The fallopian tube exhibit changes during the estrous cycle.

The fallopian tube wall:-

The wall of the fallopian tube consists of the following layers:-

- 1- Tunica serosa
- 2- Tunica muscularis
- 3- Tunica mucosa

1. The tunica serosa:- This is the outer most layer of the fallopian tube. The serosa consists of a thin layer of loose connective tissue covered by a single layer of squamous epithelium (mesothelium). This layer is well vascularized. The cell type of the serosa are the same as those commonly found in typical loose connective tissues, mast cells occurs in a relatively high number. Smooth muscle fiber are present.

2. The tunica muscularis:- The tunica muscularis lies inside the serosa and forms the middle or muscular coat of the fallopian tube. It consists of definite layers of smooth muscle fibers. The thickness of this layer varies in different segment of the fallopian tube generally being thickest in the isthmus (Isth). The isthmus has thin outer longitudinal muscle layer and a thick inner layer of circular muscles. At the junction of the oviduct and uterus, the oviducal muscle merge partly with the uterine muscles.

3. The tunica mucosa:- This is the inner most layer of the fallopian tube. It consists of a layer of lamina propria and lamina epithelialis mucosal muscle fibers are not present.

The lamina propria forms the frame work of the mucosal folds (mufol.) in the oviduct. The folds are branching longitudinal ridges. The degree of folding varies among the segment of the fallopian tube. The number of mucosal folds, their relative height and the complexity of branching decreases from anterior to posterior end of the tube. Folds are most pronounced in preampulla (Pamp) and ampulla (Amp) (Fig. 6) they are longitudinal, medium in height and complex in branching. The criterion for complex branching is that the primary longitudinal mucosal folds (Lmucfol.) from one or more prominent secondary

branches (Secbr) (Fig. 14). In isthmus and uterotubal junction the folds are longitudinal, low and simple in complexity of branching (Fig. 12, 13). The infundibulum (infid) exhibits complex branching of the primary mucosal folds which fuses to one another resulting in formation of diverticula of various sizes which look gland like in cross section (Fig. 14). These mucosal folds are called fimbriae (fimb).

The lamina epithelialis is dominated by four morphologically distinct types of cells:

1- Ciliated cell (Cicel), 2- Secretory cell (Sec cel), 3- Peg cells (Pe cel), 4- Non ciliated cells (Ncicel) (Plate VI).

1. Ciliated cells:- The ciliated cells are present in preampulla, ampulla and isthmus. The number of these cells is greater in the preampulla and ampulla (Fig. 15). The nucleus is basophilic, stains lightly with haemotoxyline, is round to oval in shape and occupies a central position in the cell, one or two nucleoli sometime being distinguished. The cytoplasm is eosinophilic.

2. Secretory cells:- The nonciliated secretory cells were observed in ampulla and isthmus (Fig. 16). The cell body is cylindrical in shape and has a large, basophilic nucleus which is elongated, centrally located 3 to 4 nucleoli are present. The cytoplasm is granular, more eosinophilic than that of adjacent ciliated cells.

3. Peg cells:- The peg cells were observed toward the posterior end of the tube. The cell was given its name because it is wedge or conical in shape (Fig. 17). The wide part of the peg forms the base of the cell while the narrow part forms the apex. The nucleus is also peg shaped, nucleoli not observed and cytoplasm is not granular.

4. Basal cells:- The basal cell is given its name because it lies along the base of the epithelium. This cell was observed in all segments of the fallopian tube (Fig.). This cell has a small round basophilic nucleus cytoplasm is not granular and weakly eosinophilic.

Oviductal segments: (Plates II, III, IV, V)

The classification of the parts of the oviduct or fallopian tube used in present study is based mainly upon the microscopic appearance of the fallopian tube illustrated by Nilsson and Reinius (1969) (Fig. 4). According to the structure of the epithelium, 4 segments of the fallopian tube may be distinguished:

1- The preampulla, 2- The ampulla, 3- The isthmus, 4- The junctura.

1. The preampulla (Fig. 4):- The preampulla comprises the fimbriae and loops 1-2. The muscular tissue consists of only

2 to 3 layers of cells, arranged mostly longitudinally. The propria is scanty and extends into high longitudinal folds. The folds meet in the middle, appreciably restricting the free space in the lumen (Fig. 5). The epithelium contains ciliated cells with few non ciliated.

2. The ampulla (Fig. 4):- The ampulla constitute loop 3 and easily distinguished on day 1. It is also the part with largest diameter. The tunica muscularis contains 1-2 cell layers, and the tunica muscularis forms longitudinal folds into the lumen. When the ampulla is distended the diameter of the ampulla is largest and tunica mucosa forms small longitudinal folds into the wide lumen (Fig. 7). When the ampulla, is not distended the diameter of the ampulla is not much large, and many mucosal folds are noticed in a narrow lumen (Fig. 6). The epithelium is dominated by non ciliated cells, but also contains numerous ciliated cells (Fig. 15).

The ampullary-isthmus junction formed by a narrow curve of the loop next to the ampulla. The opening into the isthmus has rather long mucosal folds filling its lumen and protruding into the ampullary lumen (Fig. 11). The epithelium of these folds consists mostly of non ciliated cells.

3. The isthmus (Fig. 4, 9):- The isthmus forms a long coiled part of the oviduct consist of the 4th to 9th coils. The muscular tissues are mainly circular longitudinal epithelial

folds occur only in the loop next to the ampulla. In the rest of the isthmus longitudinal folds are very less, but low circular folds have present. The width of the luman varies among the loop. The isthmus consist mainly non-ciliated cells, ciliated cells are very less in number.(Figs. 10, 11.)

4. The junctura (Junt.) (Fig. 4, 12):- The junctura constitutes the last loop of the exteramural and interamural portions of the oviduct. The muscular tissue form an even thicker coat than the isthmus and consists mostly of circularly arranged cells. The lamina propria is a dense, fibrous tissue and forms a frame work of rather high longitudinal folds along the junctura (Fig. 13). Ciliated cells are absent in the junctura. The non ciliated cells are present. The cytoplasm is rather dense. The nucleus, situated in the middle third of the cell. On days 3 to 4 the non ciliated cells of the junctura are similar to the epithelial cells of the uterus.

Cyclic changes of fallopian tube,-

The pattern frequency and amplitude of oviducal contraction vary significantly, along the length of the oviduct and during the reproductive cycle. The contractile activity is greatest at or about the time of ovulation. There is gradual increase in activity during the follicular phase (estrus) and decrease during the luteal phase (diestrus) of the reproductive cycle. It indicate that the changes in fallopian tube muscular

activity are regulated by ovarian hormonal control. The secretory activity of the tubal epithelium is enhanced during the diestrus of the ovarian cycle. Ciliated cells of the ampulla changed in height and number during the diestrus. Peg cells were not present in all cycle of the estrous. There was no cyclic variation was observed in basal cells.

EFFECT OF COPPER-INTRA-UTERINE CONTRACEPTIVE DEVICE ON THE
FALLOPIAN-TUBE

Among the experimental animals, the rat has been most intensively used for the study of the biologic action of an intra-uterine foreign body. An antifertility effect occurs which is reversible upon removal of the foreign body. In the present study copper wire has been used as intra-uterine contraceptive.

The present study was designed to evaluate the effect of intra uterine copper on the histology of the fallopian tube. No difference was observed in the histologic section of the control and deviced fallopian tube. It shows that there is no effect on Cu-IUD on the histology of the fallopian tube.

DISCUSSION

The fallopian tube provides suitable systems to support the varied processes associated with the initiation of new life. Among these are fertilization and the events which precede it, and sperm and ovum transport. The pattern frequency and amplitude of oviducal constriction vary significantly along the length of the oviduct and during the reproductive cycle. There is significant morphological and histological changes during the estrus and diestrus. Mercier (1947); Hammond (1927) and Rowan (1938) suggested, light as the stimuli for the initiation of reproductive activity, which acts through the eye and optic nerve and the pituitary gland to secrete gonadotrophic hormones. In addition to supplying hormones that initiate female reproductive activity the pituitary gland keeps the estrous cycle going. The rats have very short cycle (lasting 4-5 days).

For convenience the oviduct is usually divided into 4 segments. Anderson (1927) has also made the classification of oviduct, according to the type of the uterus. The fallopian tube consists of 3 layers these are serosa, muscularis mucosa and mucosa. Studies on oviducal muscular activity has been reviewed by Boling and Black (1969). The number, height and complexity of branching of the mucosal folds which project into

the lumen of the oviduct varies among the species and among the segment of the tube. Johnson and Foley (1974) observed the seven general patterns of mucosal folding in the isthmus, ampulla and infundibulum of each of the species they studied. Observation of the oviducal epithelium revealed for morphologically distinct type of the cells. 1- Ciliated cells, 2- Secretory cells, 3- Peg cells, 4- Basal cells. The ciliated cells occur in isthmus, ampulla and infundibulum. The literature contains conflicting reports on the cycling changes of ciliated cells. A change in height of these cells has been reported by Snyder (1923) in the pig, by Westman (1930, 1934) in the rhesus monkey and by Novak and Everett (1928) and Snyder (1923, 1924) in the human. In contrast no change in cell height was observed by Allen (1922) in the mouse nor by Hashimoto et al. (1959) in the human. The function of the ciliated cells in gamete transport has been reviewed in papers by Westman (1946) and Blandau (1961, 1969). Most investigator agree that the ciliated epithelium of the oviduct function in ovum transport, but that muscular contraction plays the primary role. Direct observations by Aden (1942b), and Blandau (1969) has conclusively demonstrated the role of ciliary action in ovum pick up by the infundibulum. Secretory cells were observed in all species studied in number approximately equal to those of ciliated cells. Hadck (1955) described significant cyclic changes in the secretory cells

of the sheep oviduct. During proestrus the cells increased in height and secretion, granules appeared within the cells. During estrus the height of the cells reaches their maximum and the secretory products were expelled into the lumen of the fallopian tube. Electron microscopic observations by Nilsson (1958) of secretory cells in the rabbit oviduct revealed that secretory granules increased in numbers during estrus.

Similar cyclic changes of the secretory cells have been noted in the rat and mouse with the electron microscopy by Nilsson and Reinius (1970). Novak and Everett (1928) claim to have recognised definite transitional stages between peg cells and secretory cells in the human and concluded that the peg cells were exhausted secretory cells. Allen (1922) described the similar occurrence in rat, but recent studies of the oviduct revealed no transitional changes between these cell types.

Cyclic variation in the morphology of the basal cells have never been observed with the light or electron microscopy.

Snyder (1923) noted that the basal or round nucleated cells occurred along the base of the human oviducal epithelium but did not comment on their functions. Pauerstein and Woodruff (1968) have studied the function of the basal cells in the human. Earlier reports show that it is a distinct cell type and that it can be morphologically distinguished from the outer cell in the epithelium.

No information is available on the effect of an IUCD on the morphology, histology, ultrastructure or histochemistry of the tubal tissue. This is also true for the tubal fluid milieu, in some study an increase in tubal motility in the presence of an IUD have been reported. In other studies the motility remained normal in woman fitted with an IUD (WHO). The biological effect of intrauterine devices (IUD) differ from species to species and no common denominator has been discovered which explains fully their antifertility action. Ovulation, egg and sperm transport, fertilization, nidation and survival of the embryo have been implicated in varying degree for different species (Segal, 1968). There is no effect of IUD on the estrous cycle or on the corpus luteum (Marston and Chang, 1964); Schunner and Davidson, 1965) and sperm transport is not altered (Marcus, 1966) upon removal of the thread, fertility is restored (Kar et al., 1967). It appears that the ova transport through the oviduct is not affected but that blastocysts degenerate or are prevented from implanting when they enter the uterine cavity at approximately day 5 (Kar et al., 1964; Margolis and Doyl, 1964). Tubal potency tests establish that the presence of IUD does not cause mechanical obstruction of the oviduct. The results of scores of investigation over the past few years have disclosed that intrauterine devices affect a wide range of reproductive processes.

BIOCHEMISTRY OF THE FALLOPIAN TUBE

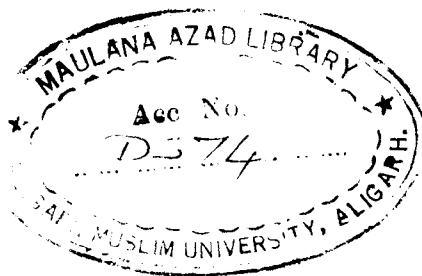
INTRODUCTION

With the development of the modern intra-uterine contraceptive devices there has been resurgence of interest in their use as a means of controlling a rapidly growing world population. The rapidly increasing use of the intra-uterine device (IUD) for family planning purposes has stimulated considerable research on its biological effects in experimental animals and human beings. Several mechanisms are probably involved in the contraceptive action of Cu-IUD. Chang and Tatum (1970) suggested that copper wire produced a local change in the intra-uterine milieu such that the blastocyst can not implant. Foreign bodies in the bicornuate uterus of the rat have a less extensive effect than they do in mice. Threads of silk, or nylon, or devices composed of materials used in manufacture of intra uterine devices for woman will prevent blastocyst nidation in the horn in which they are inserted but have no antifertility effect on the contralateral untreated horn (Doyle and Margolis, 1963-64). The foreign body must lie freely in the endometrial cavity and the endometrium must be pierced; if the thread lies only in the myometrium, it has no antifertility effect (Skeilberger et al., 1966). There appears to be no effect on the estrous cycle or on the corpus luteum (Chang, 1965) and sperm transport is not

altered (Marcus, 1966) upon removal of the thread fertility is restored (Kar et al., 1967). The ova transport through the oviduct is not affected. The biochemical composition of the tubal fluid does not show any noteworthy alteration in the presence of an intra-uterine foreign body in rabbit (Kar et al., 1965). Particular general interest in the possibility that IUD may accelerate transport of ova through the oviduct. A unique study in rabbit has shown that an intra-tubal thread may speed ova passage (Chang, 1966 and Kar et al., 1966). Other studies with intra uterine threads indicate a normal rate of passage of tubal ova (Ishihama et al., 1966). The evidence is that the IUD increases the rate of tubal transport in monkeys that have been ovulated artificially with exogenous gonadotropins and artificially inseminated (Mastroianni et al., 1964; Mastroianni, et al., 1966). In this situation, ova are transported through the tube in several hours instead of the 3 to 4 days normally required. Tubal potency tests establish that the presence of an IUD does not cause mechanical obstruction of the oviduct (Seigler and Hillman, 1964). Normal motile sperm have been found in fallopian tube and uteri of woman bearing IUD's at least 24 hours after coitus (Malkani and Sujon, 1964). Although recent work indicates that the number of sperm may be fewer than expected.

Significant biochemical changes have been observed in the fallopian tube. Kar et al. (1965) reported an increase in lactic acid content of the tube and consider it important in the development of the ovum. Moghissi (1971) and Fergelson (1972) have studied the protein patterns of the fallopian tubal fluid. Friedly and Rosin (1975) have observed histochemically and biochemically carbonic anhydrase. But there is very little information available on the biochemistry of the fallopian tube in the presence of copper intra-uterine foreign bodies. Most of the studies are devoted either to uterus or to vagina.

The present study has been undertaken to demonstrate the zygote of Cu-IUFB on the level of glycogen and protein in the fallopian tube of the albino rats.



MATERIAL AND METHODS

Three months old female albino rats were used in the present study. Rats were kept under normal laboratory conditions. Normal cycling females were picked up and now cycling females discarded. Under anaesthesia the reproductive tract was, exposed through a small midventral incision. Unilateral insertion of copper wire as IUB was made in the left uterine cornu, while contralateral cornu served as control. After two weeks of recovery period, the rats were sacrificed in the estrus and diestrus phases of reproductive cycle. The fallopian tube was removed surgically and placed in cold Robinson's medium. After trimming off of the fat and adhering connective tissues, the tube was processed for biochemical estimation of glycogen and protein.

Estimation of glycogen:

Glycogen was estimated by the method of Dubois et al. (1951). 30-40 mg of the tissue were dissolved separately in 30% KOH. After cooling sodium sulphate and 95% ethyl alcohol were added. The samples were left for overnight at room temperature. Then the samples were centrifuged at 4,000 rpm for 30 minutes. To the precipitate 80% of phenol and lately 5 ml of concentrated sulphuric acid was added for colour

developing. The developed colour was read against a blank on spectrophotometer at 490 nm. A calibration curve for glycogen was prepared by reading against standard. The reading obtained for unknown solution was read against the calibration curve, which gave reading for glycogen against the fallopian tube.

Estimation of protein:

Protein was estimated by the method of Lowry et al. (1951). 70-80 mg of the tissue were taken and homogenised in all glass homogeniser in the presence of 2 ml of 5% perchloric acid (PCA). The homogenate centrifuged and the resultant supernatant fraction discarded. Precipitate washed twice with 2 ml of 0.6% normal PCA to remove the acid soluble nucleotides. The precipitate further washed with 2 ml of 80% ethanol, 100% ethanol, chloroform and ether. The supernatant from above washing discarded. The precipitate was dissolved in 3 ml of 0.2 N - NaOH, incubated at 37°C for 16-18 hours, centrifuged and washed with 0.6 N PCA. The final precipitate obtained were dissolved in 1% N NaOH, added 1 ml freshly prepared reagent and 0.1 ml Follin's reagent for developing colour. The colour developed was read against a blank on spectrophotometer at 500 nm. A calibration curve for protein was prepared and the reading obtained for unknown solutions were read against calibration curve, which gave reading for total protein in the tissue used.

Table

Group	No. of animals	GLYCOGEN μ / 100 mg tissue		PROTEIN μ / 100 mg tissue	
		Estrus	Diestrus	Estrus	Diestrus
Control Ut. cornu	30	2140 \pm 8.0	219.0 \pm 5.7	98.5 \pm 5.4	93.0 \pm 3.7
Treated Ut. cornu	30	212.5 \pm 9.5	220.5 \pm 8.9	96.0 \pm 5.2	94.0 \pm 4.2

RESULTS

The result presented in the table clearly show that Cu-IUFB has no significant effect on the biochemical composition of glycogen and protein, are more or less similar in the fallopian tube of the control as well as of the Cu-IUFB bearing side. Further no significant change was observed in the level of glycogen and protein in estrus and diestrus phases of the cycle.

In estrus, glycogen level was 214.0 μ / 100 mg tissue in the control and 212.5 μ / 100 mg tissue in the fallopian tube of treated side in contrast to the 219.0 μ / 100 mg tissue and 220.5 μ / 100 mg tissue respectively in diestrus condition. The protein level in fallopian tube of control and treated side is 98.5 and 96.0 μ / 100 mg tissue in comparison to 93.0 and 94.0 μ / 100 mg tissue in diestrus condition.

DISCUSSION

The antifertility effect of copper intra-uterine device seems to be due to a local change in the intra-uterine environment such that the blastocyst does not implant (Chang and Tatum, 1970). The fertility effect is confined to the uterine cornu bearing the foreign body. Corfman and Segal (1968) have reported that the treated animals have normal estrous cycle and normal corpus luteum function, ovulation, sperm transport, fertilization and zygote transport through the fallopian tube occur normally. It has been observed by Kar et al. (1964) that the biochemical and enzymological responses to intra-uterine devices occur only in the treated cornu, while in control cornu remains as usual, Cu-IUFB markedly increase the glycogen and protein content of the treated cornu as compared to the control cornu (Murad et al., 1976). It has been observed that the presence of Cu-IUD has significant effect on the level of glycogen and protein in lung, kidney, pancreas, spleen and urinary bladder (Hasan et al., 1976). It has been suggested that the effect of IUD is localized and of neurogenic origin and interplay through hypothalamo-hypophyseal centre (WHO, 1968). This hypothesis was further upheld by Tatum (1973) who reported that copper content of liver, lung etc. remain unaltered in the presence of Cu-IUD, signifying its local effects on the uterine cornu bearing it.

SUMMARY

The organs of reproductive system of female rat divided into the essential and accessory. The essential sex organs are gonads or ovaries, these are two in number. The essential sex organs are paired fallopian tube, paired uterine horns, cervixes, vagina and external genitalia.

1. The rat fallopian tube is connected with ovary by an ovarian bursa, it is convoluted structure consisting almost 8-10 loops. The last portion of the fallopian tube runs intramurally in uterus.

2. According to the structure of the epithelium, 4 segments of the fallopian tube may be distinguished, the preampulla, the ampulla, the isthmus and the junctura.

3. The oviductal wall consists following layers:-

- a- tunica serosa
- b- tunica muscularis
- c- tunica mucosa

4. Observation of the oviductal epithelium with the light microscope revealed four morphologically distinct types of cells:-

a - ciliated, b - basal cells, c- secretory, d- peg cells.

5. Ciliated cells occur mostly in preampulla and ampulla although they may be found in isthmus and junctura.
6. The basal secretory cells are more frequent than the ciliated cells in the isthmic portion of the fallopian tube.
7. Peg cells are mostly present in posterior end of the fallopian tube.
8. Basal cells observed in all segments of the tube.
9. Cyclic variations were observed in both ciliated and secretory cells.
10. The activity of the ciliated cells was greater during the estrus phase of cycle than diestrus phase.
11. The secretory activity of the tubal epithelium was observed to be enhanced during the diestrus phase of the cycle.
12. No significant difference were noted in the histologic section of the control and deviced fallopian tube it shows that there is no effect of Cu-IUD on the histology of the fallopian tube.
13. It is being observed that the Cu-IUFB does not affect the biochemical composition of fallopian tube to any notable extent. The level of glycogen and protein content in the fallopian tube connected with Cu-IUFB bearing horn were similar to that of the control horn.

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LIST OF ABBREVIATIONS

Amp	Ampulla
Amp tub ute	Ampulla tubae uterinae
Amp isth jun	Ampullary isthmio junction
Cer	Cervix
Cicel	Ciliated cell
Ext joviut	External junction of oviduct and uterus
Ext muca	Extra mural cast
Flt	Fallopian tube
Infid	Infundibulum
Int joviut	Internal junction of oviduct and uterus
Int muca	Intra mural cast
Isth tub ute	Isthmus tubae uterinae
Jnct	Junctura
Kd	Kidney
Lu	Lumen
Mu fol	Mucosal fold
Ncicl	Non ciliated cells
Os	Ostium
Os tub ute	Ostium abdominal tubae uterinae
Ov	Ovary
Pa ute	Pars uterina
Pamp	Preampulla

Pe cel

Peg cell

Sec br

Secondary branches

Sec cel

Secretory cell

Secn

secretion

Ugs

Urogenital sinus

Ur br

Urinary bladder

Uth

Urethra

Ut H

Uterine horn

PLATE I

Fig. 1. Female reproductive system

Fig. 2. The uterine horns and cervixes.

PLATE I

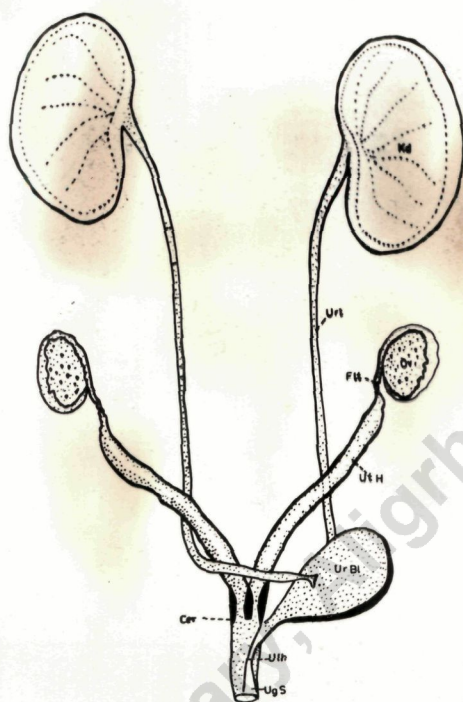


Fig.1

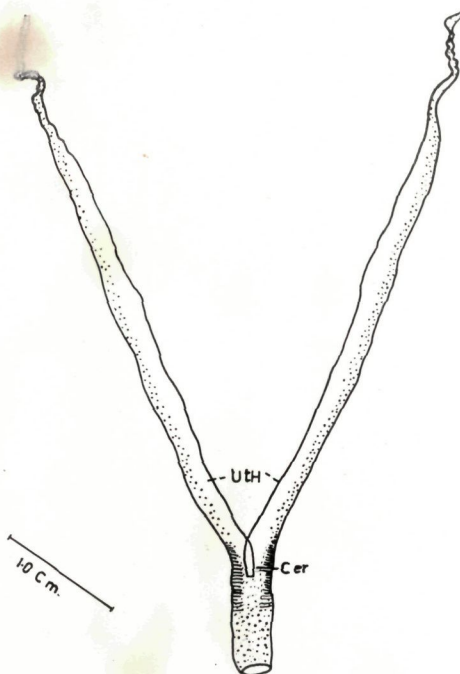


Fig.2

PLATE II

Fig. 3. Fallopian tube

Fig. 4. Schematic drawing of the fallopian tube.

PLATE II

